EXERCISE

2 Care and Use of the Compound Light Microscope

Time Estimates for Completing This Lab

The activities in this laboratory exercise can be completed in 2 to 2.5 hours. Extra time will be required to complete the review sheets at the end, or they may be assigned as homework. Times listed are only estimates.

Activity 2.1: Learning the Parts of a Light Microscope
20 minutes

Activity 2.2: Viewing a Specimen with a Compound Microscope
30 minutes

Activity 2.3: Inversion of Image: Viewing the Letter *e*
20 minutes

Activity 2.4: Perceiving Depth of Field
20 minutes

Activity 2.5: Determining the Diameter of the Field of View
30 minutes

List of Materials

This list of materials shows the quantities needed for a standard 24-seat lab, with six tables and four seats at each table. [*Note:* Other than the microscopes, if resources or space is limited, this lab could be set up with one set of all other items per pair of students, or even one set per table. This would reduce the quantities needed from 24 of each item to 12 of each if shared in pairs, or to 6 of each if shared per table.]

- 24 compound light microscopes
- 24 sets of prepared microscope slides of various tissues
- 24 prepared microscope slides of the letter “e”
- 24 prepared microscope slides of intersecting colored threads
- 24 clear millimeter rulers
- 24 pads of lens paper

To Do in Advance

√ ____ **1.** Set up equipment and supplies on all laboratory tables.

√ ____ **2.** Familiarize yourself with the particular model (or models) of microscope your students will be using.

√ ____ **3.** Be sure all microscopes are clean and the lowest power objective is in place.

√ ____ **4.** Perform Activity 2.4 in advance so you know the correct order of the threads on the particular slides that your students are using.

Tips and Trouble Spots

Introduction

Take time at the beginning of your laboratory session to discuss the section "Care and Use of the Compound Microscope" with your students. This step can help you avoid many problems, including potential damage to both the microscopes and the slides.

You may begin by polling the class to see which students do and do not have microscope experience. Those lacking experience will require more hands-on guidance from you while doing the exercise. If you do not regularly check on your students' progress, they may skip steps or not realize they are not using the equipment appropriately. If many students are lacking experience, it may be worthwhile to provide a class demonstration, showing your students the location of the specific parts of the microscope.

Activity 2.1: Learning the Parts of a Light Microscope

Learning Outcome: Identify the parts of a compound light microscope and explain their functions.

Be familiar with the microscopes in your laboratory. They may not have a mechanical stage. Instead, they may have two movable stage clips. If so, these should be swung out to the side, the slide positioned between them, then the clips rotated in to secure the slide. Students should not lift the stage clips as they will become bent and useless with time.

If more than one model of microscope is in use in your lab, be sure students are aware of any differences and become familiar with them.

Objective lenses may have two numbers stamped on them. The magnification may not have an "X," but typical magnifications are 3X or 4X for a scanning objective, 10X for low, 40X to 45X for high, and 100X for oil immersion. Other numbers, if present, are usually DIN numbers, which refer to the optical standards used for the lens.

Students often have trouble distinguishing between the iris diaphragm and the condenser lens. They may need assistance with this.

Activity 2.2: Viewing a Specimen with a Compound Microscope

Learning Outcome: Demonstrate the proper method for viewing a specimen with the compound microscope.

Stress to your students the importance of always starting on the lowest power because they can more easily locate the specimen and should not (usually) be able to plow through the slide. Be sure they know to only use the fine adjustment knob when moving to high power so they do not damage the slide.

Students working with a binocular microscope may need assistance in positioning the two eyepieces so they get a single image. Watch for students closing one eye and using only one eyepiece and help them make the needed adjustment.

Activity 2.3: Inversion of Image: Viewing the Letter *e*

Learning Outcome: Describe the principal of inversion of images.

Students may be unclear on how to position the slide on the stage. Tell them to place the slide so the letter "e" appears as it would if they were reading their book.

Activity 2.4: Perceiving Depth of Field

Learning Outcome: Understand the concept of depth of field.

Students should be reminded to only use the fine adjustment knob when they move to high power and to proceed slowly and cautiously. Too often they forget this during this activity and crack the slide while exploring the order of the three threads.

Colored thread slides from different manufacturers may have the threads in different orders. Be sure to check the slides your students will be using in advance so you know the correct order of the threads from top to bottom.

Activity 2.5: Determining the Diameter of the Field of View

Learning Outcome: Measure the diameter of the field of view and estimate the size of structures in a tissue section.

Students may be unclear on how to place the ruler through the field of view. Draw or show an example on the screen or a board so students see the importance of centering the scale to measure the diameter, and the importance of placing a number at the left edge. Students also need to be reminded that if the diameter falls between marks on the ruler, they estimate the additional distance, not just go by the last whole number. Thinner rulers are best for this activity.

Students get frustrated when moving to high power as they mostly see part of one of the number marker lines. It becomes almost impossible to estimate millimeters with high power, but that helps reinforce the need for other units, such as micrometers, for microscopic measurement.

Additional Student Engagement Ideas

- Even though students will be testing how magnification affects the field of view, they do not always understand the concept because they may not really understand what they are looking at through the microscope. As an alternative or an additional activity, provide each student with three index cards. Using the small rulers, have them cut a circle about 20 mm in diameter from the center of one card, which they will use for viewing. On the second card, have them mark a box 35 mm × 35 mm square, and draw a picture that fills the box—it can be of anything with which they are familiar. On the third card, have them mark a box twice that size (70 mm × 70 mm) and redraw their picture, enlarging it to fill the larger box. *(Having them measure in millimeters will reinforce that dimension when they do Activity 2.5.)* When done, have them place the "viewing" card over the second card and slide it around, noticing how much of their drawing is visible. Have them repeat this on their enlarged drawing. They will easily see the change in the field of view that results from magnification, and they should realize why it is beneficial to always begin with the microscope on low power to get the specimen centered properly before going to higher power.

- Although students will gain an understanding of depth of field and field of view from this exercise, they often struggle with what they see through a microscope because they don't understand how slides are prepared. Several short videos are available online that explain the process, but students will understand it better if they learn firsthand. An easy way to convey this information is to prepare in advance a gelatin mold with various objects embedded in it. Use a light-colored commercial gelatin and follow the box instructions. Once the gelatin is partially set, add an assortment of objects, such as pieces of fruit, candy, or straws; be creative. Unmold the gelatin and let the class examine it. Then have them use a sharp knife to make thin slices through it, like cutting a loaf of bread. As they examine the slices, they will begin to appreciate how slides are sectioned for microscopy. That will help them understand how the same objects can look quite different through a microscope, depending on the angle of sectioning.

Exercise 2 Answers

Pre-Lab Quiz

1. a
2. b
3. True
4. False (you should only use the fine adjustment knob to bring it into focus at this point)
5. field of view
6. objective lenses
7. condenser lens
8. d
9. c
10. a

Activity 2.1 Answers

A 1. ▸ Students should see the amount of light changing.

2. ▸ One knob moves the stage forward and backward, and the other moves the stage left and right.

6. ▸ Turning the coarse adjustment knob clockwise lowers the stage; turning the knob counterclockwise raises the stage.

B ▸ 10X • 40X = 400X total magnification

▸ MAKING CONNECTIONS: The fine adjustment knob is designed for very precise and very small changes in focus to bring an already focused object into the sharpest focus, and thus, one complete revolution will barely move the stage. In contrast, the coarse adjustment knob is designed for rapid initial focusing and thus produces much more movement per revolution.

Activity 2.2 Answers

C 3. ▸ Moving the condenser lens will increase or decrease both the total illumination and the resolution of the field of view.

D 4. ▸ Moving to higher magnification decreases the overall size of the field of view.

▸ Moving to higher magnification decreases the field of view, and thus less light is seen.

- MAKING CONNECTIONS: The entire specimen is illuminated from below. Only the light passing through the particular part of the specimen in the field of view reaches the eye. When moving to higher power, the field of view is reduced. Less of the specimen is in the field of view, so less light is transmitted.

Activity 2.3 Answers

A 1. ▸ e (direct viewing)

2. ▸ ə (under low power)

3. ▸ The image is upside down and reversed.

4. ▸ As the slide moves to the right, the image appears to move left.

▸ As the slide moves away from you, the image appears to move toward you.

- MAKING CONNECTIONS: The image appears to be inverted when viewed through the microscope—it is upside down and also reversed from side to side. When the slide is moved in one direction, through the microscope it appears to be moving in the opposite direction.

Activity 2.4 Answers

A 5. ▸ Due to manufacturer variations, the order of the threads will vary depending on what specific slides you provide for the students to view.

- MAKING CONNECTIONS: Depth of field decreases as magnification increases. The image is magnified, but the actual diameter of the tube through which the image is viewed is unchanged, so less of it is visible within that space.

Activity 2.5 Answers

A 6. ▸ Magnification data will depend on your particular microscopes, but the most common values are listed below.

Type of objective lens	Magnification of objective lens	Magnification of ocular lens	Total magnification	Diameter of the field of view (mm)
Scanning	*4X*	*10X*	*40X*	*About 4–5 mm*
Low power	*10X*	*10X*	*100X*	*About 2 mm*
High power	*40X*	*10X*	*400X*	*About 0.4 mm*

7. ▸ The diameter decreases as the magnification increases.

B 1. ▸ The magnification of the ocular lens is 10X. If you are using the scanning objective lens, the total magnification will be 10X times 4X, which equals 40X.

3. ▸ Answers vary with the structures selected to measure and the individual estimations each student makes.

7. ▸ Answers will vary based on the previous question.

8. ▸ Answers will vary based on the previous two questions.

- MAKING CONNECTIONS: Answers will vary based on the specific data the students collect. If they performed the steps and did the math correctly, their results should support the relationship.

Looking Back

Most of the events and functions that occur in the human body begin at the cell and tissue level. Examining microscopic anatomy provides a constant reminder of this, and as students visualize microstructures, they will be able to understand the physiological processes better. For example, by examining the microstructure of blood vessels, students can see that only capillaries have a thin enough wall to allow exchange.

Answers to Review Sheet

1. e. Ocular lenses
2. b. Objective lenses
3. f. Coarse and fine adjustment knobs
4. d. Microscope head
5. c. Nosepiece
6. a. Mechanical stage
7. g. Iris diaphragm
8. h. Substage light
9. With a parfocal microscope, initial focusing is done rapidly with the scanning objective or low power objective, where more of the specimen is visible (larger field of view) so that it can easily be centered. Once the specimen is centered and in focus at low magnification, the magnification can be increased and the specimen remains almost in focus so that only the fine adjustment knob needs to be used. This makes it easier to get the desired object centered and in focus, and it minimizes the risk of damage to the microscope and the slides.
10. Resolving power is the ability to distinguish close objects as separate and distinct.
11. Working distance is the distance between the slide and the objective lens.
12. The field of view refers to the area of the slide that is visible when viewed through the microscope.
13.

Low magnification	**High magnification**
Larger field of view	*Smaller field of view*
Greater depth of field	*Smaller depth of field*
More light transmitted	*Less light transmitted*
Less detail visible	*More detail visible*

14. The image will be upside down and reversed left to right.
15. The depth of field refers to the thickness or depth of a specimen that is currently in focus.
16. To determine the diameter of the field of view, use the equation $M_1D_1 = M_2D_2$.

    $M_1D_1 = 40X \bullet 5.0\text{ mm} = 200\text{ mm}$
    Thus M_2D_2 must also equal 200 mm. $M_2 = 100X$, so D_2 must equal 2.0 mm.

    The diameter of the field of view at 100X is 2.0 mm.

